The importance of characterising critical reagents with a focus on bio-conjugated reagents

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Overview

- **Introduction:**
  - Critical reagents
  - Bio-conjugated reagents
- **Case studies**
  - Sulfotag conjugated novel therapeutic antibody
  - Bio-conjugated viral capsid
- **Comparison of characterisation methods**

Introduction

Critical reagents - proven to be essential to the performance of immunoassays

"As a general statement, assay reagents are deemed to be critical, if they have a direct impact on the assay performance due to their quality, structure, nature or specificity."

- Pihl S, van der Strate BW, Golob M, *et al*. EBF recommendation on practical management of critical reagents for antidrug antibody ligand-binding assays. Bioanalysis. 2019 Oct;11(19):1787-1798.

- Using a different batch may have consequences on critical assay parameters
  - Sensitivity
  - Assay range
  - Drug/target tolerance
  - Selectivity

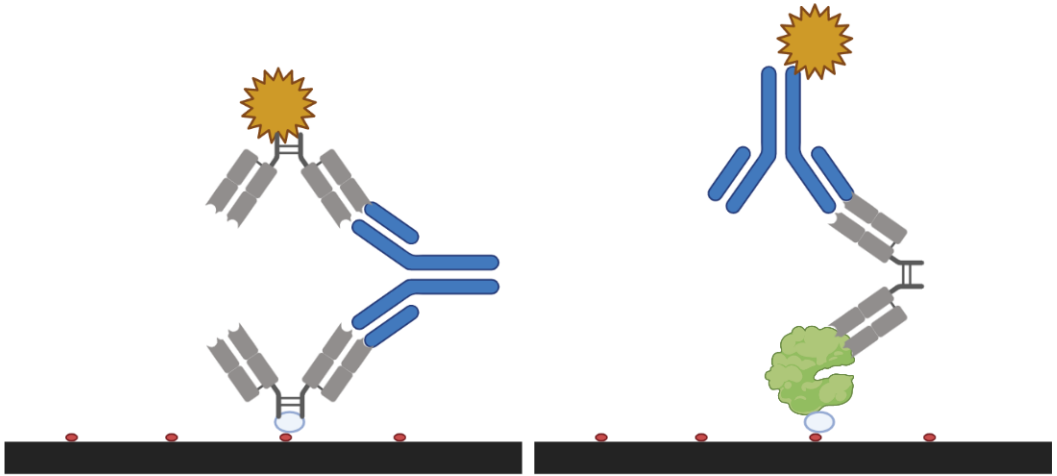
Introduction

Immunogenicity

Bridging format

PK

Sandwich format



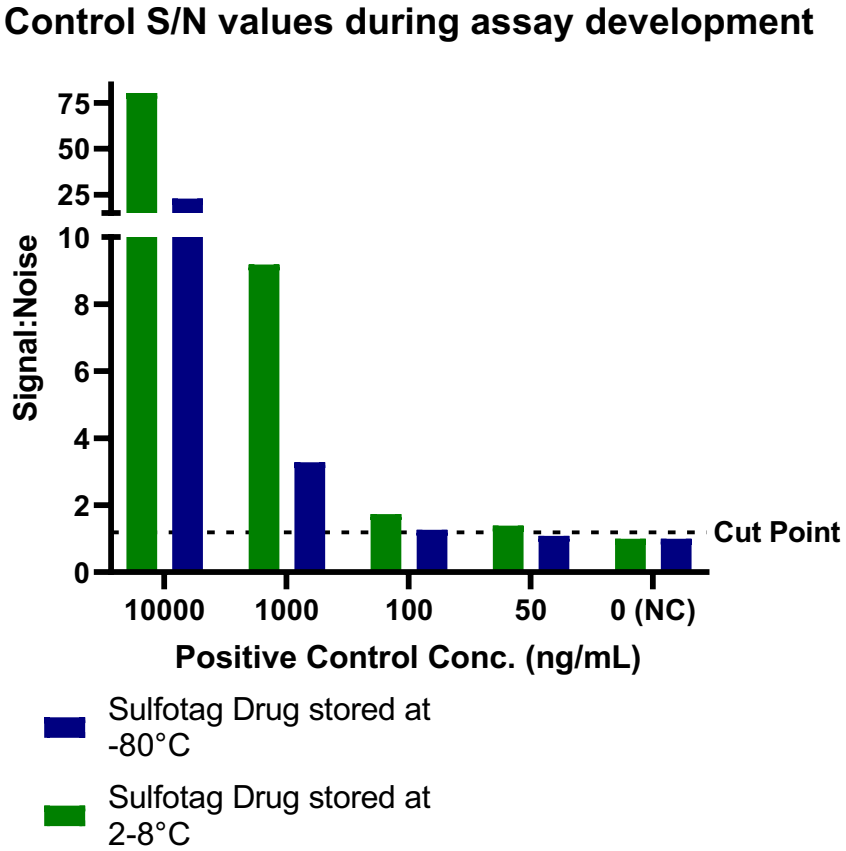
Introduction

- Batch to batch variation in bio-conjugated reagents is present, even with a well defined bio-conjugation procedure

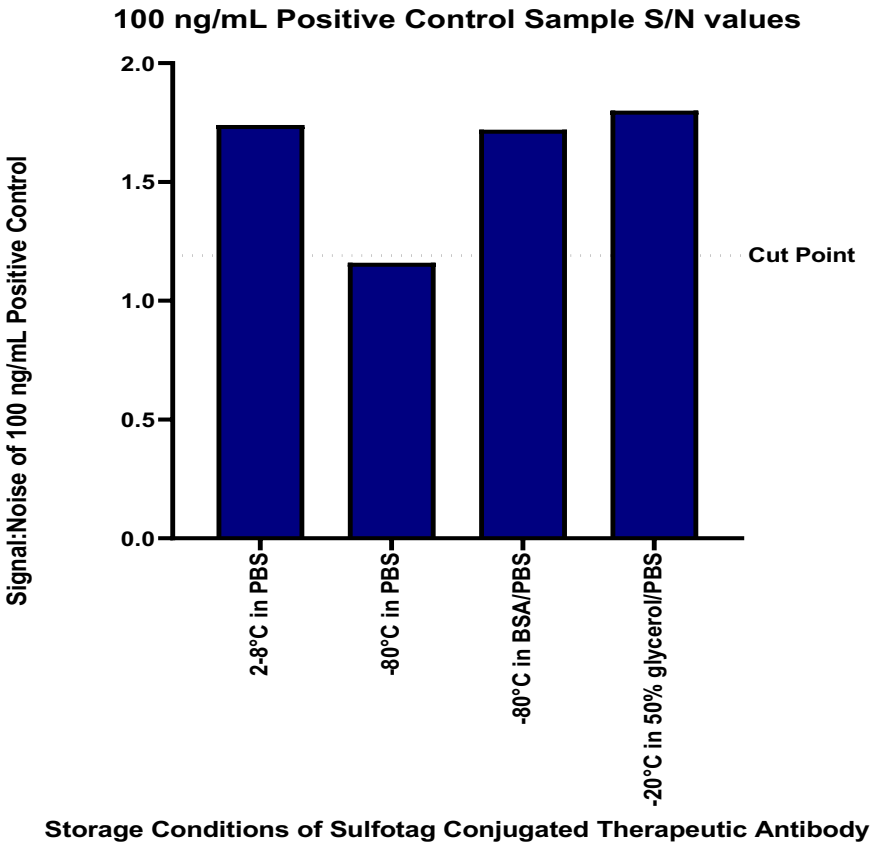
- Effective ways of characterising critical reagents are required:
  - Troubleshooting
  - Determining reagent stability
  - Comparison of new and old reagents

Case Studies

Case study one: Sulfotag conjugated Novel Therapeutic Antibody



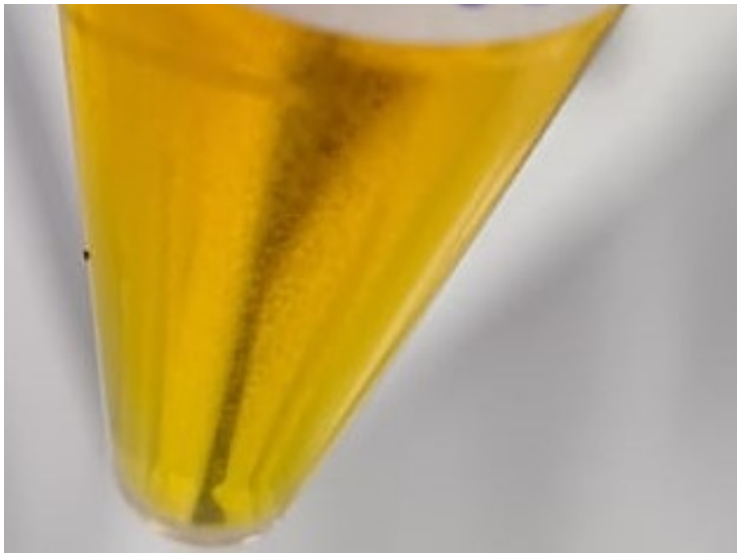
Case study one



Case study one

A new batch of drug was received and bio-conjugated

- **Precipitation was observed in the reagent**
- **Assay sensitivity was reduced**

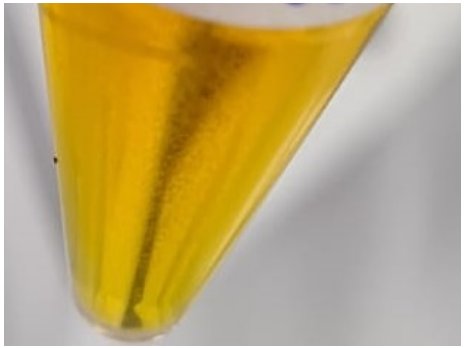


Case study one

A new batch of drug was received and bio-conjugated

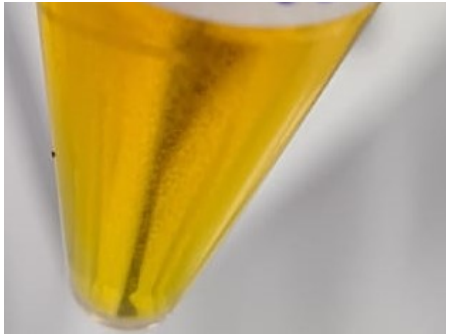
- Precipitation was observed in the reagent
- Assay sensitivity was reduced

Characterisation parameter	Original bio-conjugated reagent	New batch of bio-conjugated reagent with precipitate
Concentration (determined by BCA assay)	2 mg/mL	1.9 mg/mL
Label to protein incorporation ratio (determined by spectrophotometry at 455 nm)	7.8	7.7

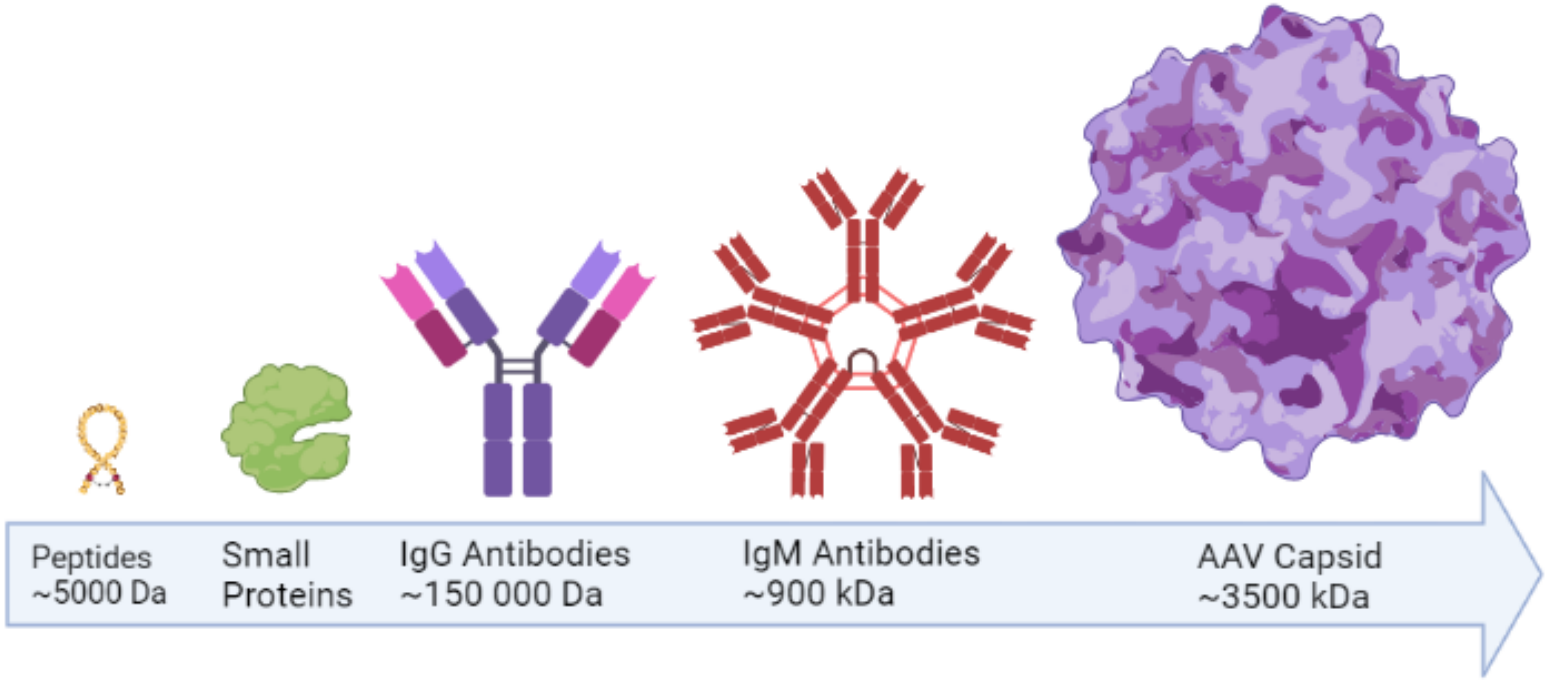


Case study one

- **Methods assessed to remove precipitation**
  - **Additional post-conjugation clean up procedures** ✗
  - **Centrifugation** ✗
  - **Filtration** ✗
  - **Bio-conjugated reagent buffer exchanged back into excipient buffer post bio-conjugation** ✓



Case study two: Bio-conjugation of AAV capsid



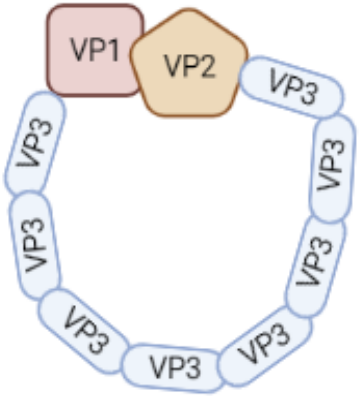
It is important to know the size of the reagent being bio-conjugated

Case study two

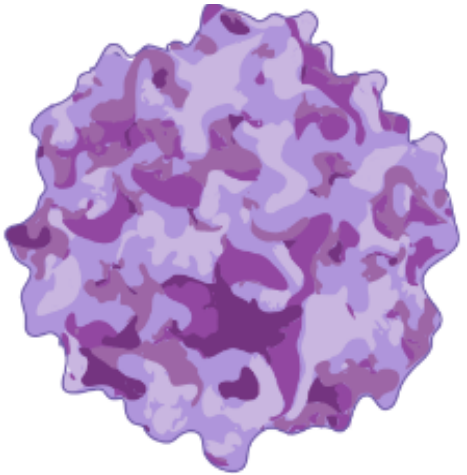
- Bio-conjugation of Adeno Associated Virus with both sulfotag and biotin
- Molecular weight and structure was initially unknown
- Appeared that very little/no biotin or sulfotag had been conjugated, however the reagent worked in the assav

Structure confirmed as:

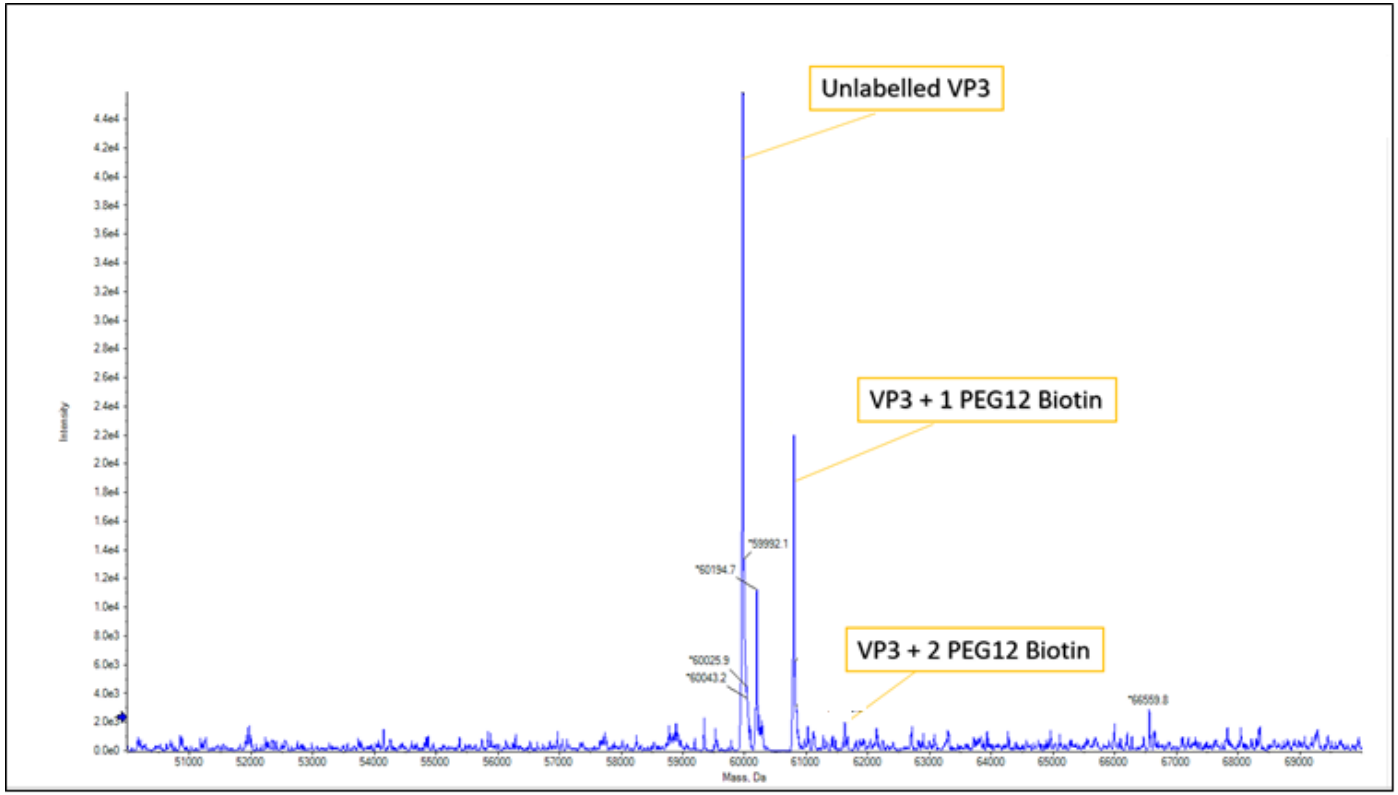
- VP1:VP2:VP3
- (1:1:8) x 60
- 3500 kDa



X 60



Case study two

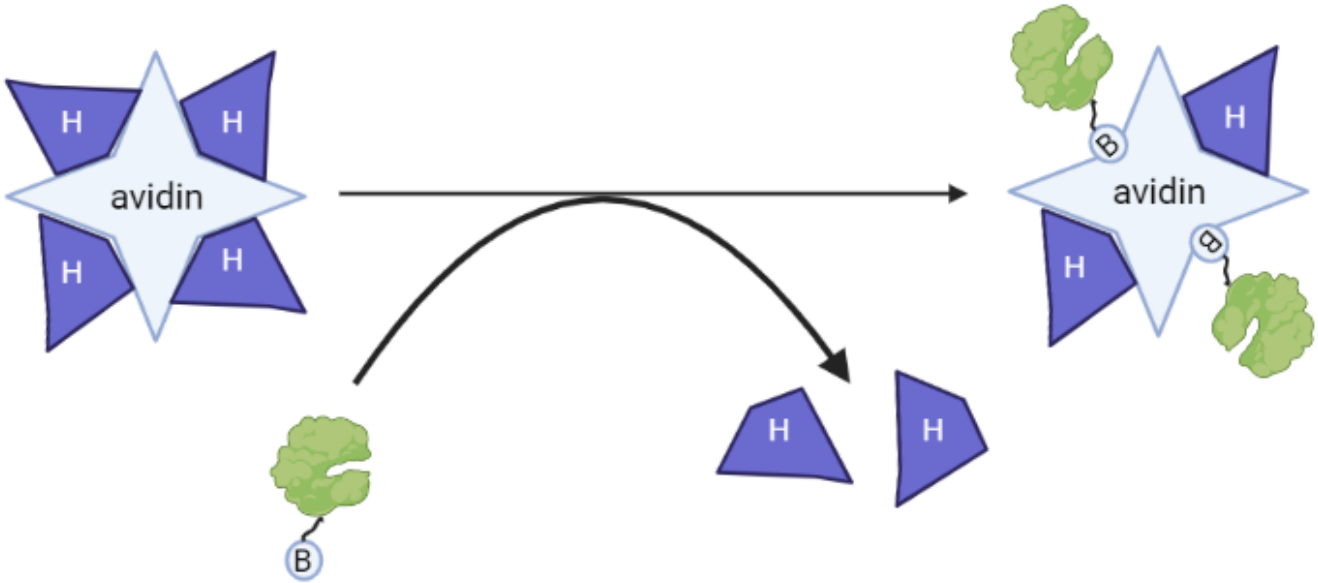


Reagent Characterisation

Comparison methods of determining incorporation ratio

- **HABA** (4'-hydroxyazobenzene-2-carboxylic acid):

HABA/avidin absorbs light at 500 nm, the biotin on biotinylated reagents displaces the HABA resulting in a decrease in absorbance



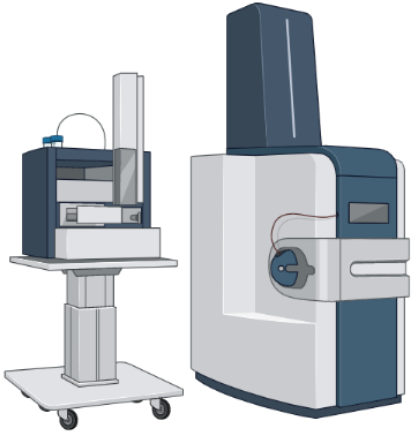
Comparison methods of determining incorporation ratio

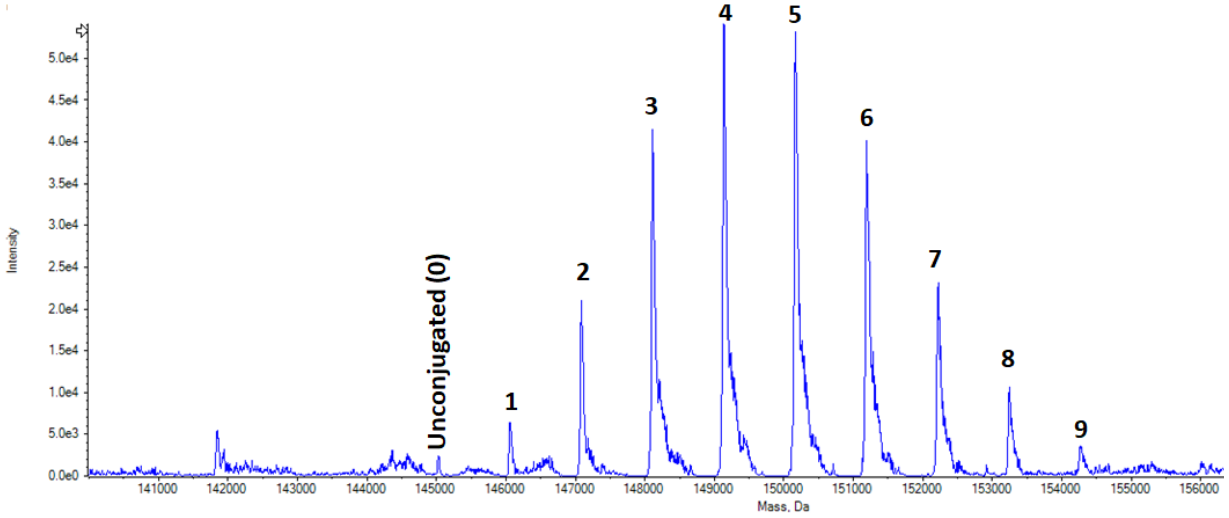
- **HABA**
- **QuantTag:**

The reagents chemically react with the biotin producing a coloured product that can be quantified by measuring absorbance at 535 nm.

Comparison methods of determining incorporation ratio

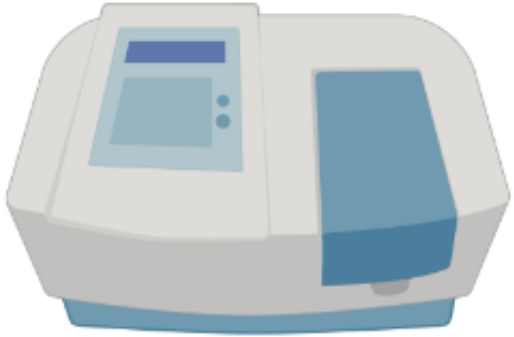
- HABA
- QuantTag
- LC-MS:





Comparison methods of determining incorporation ratio

- HABA
- QuantTag
- LC-MS
- Spectrophotometer



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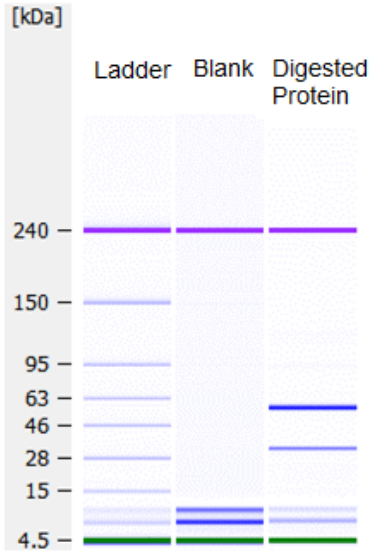

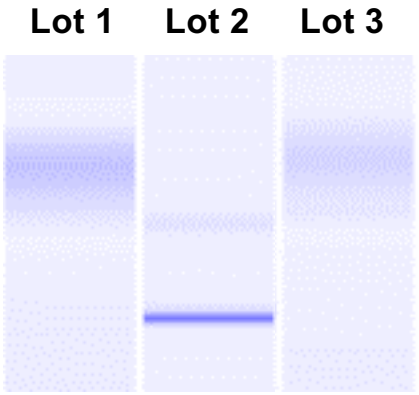

- **Bioanalyzer:**

Microfluidics-based automated electrophoresis

Comparison of methods to determine incorporation ratio

	Average biotin/sulfotag Incorporation result			
Analyte	**LC-MS**	**QuanTag**	**HABA**	**Spectrophotometry result at 455 nm**
Biotinylated IgG therapeutic (CR20)	3	9.2	6.5	N/A
Sulfotagged IgG therapeutic (CR10)	2.3	N/A	N/A	3.5
Sulfotagged IgG therapeutic (CR20)	4.4	N/A	N/A	5.8

Summary

- It is important to determine appropriate suitable storage conditions for bio-conjugated critical reagents
- It is critical to gather information about the reagent to be bio-conjugated e.g. molecular weight, structure and formulation buffer
- It is critical to characterise bio-conjugated critical reagents
  - To ensure batches are comparable
  - Troubleshooting
- Different characterisation methods available

Acknowledgements and references

Acknowledgements:

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- LGC, Szabolcs Szarka & LGC LC-MS Bioanalysis Department
- Sponsors

References:

- Pihl S, van der Strate BW, Golob M, et al. EBF recommendation on practical management of critical reagents for antidrug antibody ligand-binding assays. Bioanalysis. 2019 Oct;11(19):1787-1798.
- Images Created on BioRender.Com

Questions?

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